1167-Pos Board B77
Macromolecular Crowding Affects the Mechanical Unfolding Forces in Titin: The Size Effect
Marisa I. Roman, Gouliang Yang.
Macromolecules can occupy a large fraction of the volume of the cell and this affects many properties of the proteins inside the cell, such as thermal stability and rates of folding. We present a study comparing the effects of the size of molecular crowders on the unfolding forces of titin. We used an atomic force microscope based single molecule method to measure the effects of the crowding on the mechanical stability of this protein. We used dextran as the crowding agent with three different molecular weights, with concentrations varying from zero to 300 grams per liter in the buffer solution. The results show that the forces that are required to unfold molecules are enhanced when high concentration of dextran molecules is added to the buffer solution and also that there is a maximun force when the crowder size is comparable to the protein.

Protein Structure Prediction & Drug Design

1168-Pos Board B78
Computational Simulation of a Beta-helical Tubular Peptide in a Lipid Bilayer
Ayat Saeedi, Anahita Kyani, Armin Madadkar-Sobhani, **Bahram Goliaei**.
Peptides having a regularly repeating pattern of L and D amino acids adopt unique structures termed beta-helix. In beta helix structure, intramolecular hydrogen bonding looks like that in parallel β-sheets. The beta helix structure has been reported for both natural peptides, like gramicidin A, and syntetic peptides such as poly (D, L-γ -benzylglutamyl). Studies have shown a short beta sheet tetraicosa-peptide (VSLGLSIAFSVAVSIAWSFARSRG, where all As are D-alanine) accept beta-helical conformation in lipid bilayer similar to gramicidin A [4], called gramicidin-like channel (GLC).
In the present work, the GLC syntetic peptide has been modeled and simulated in different orientations with respect to a lipid bilayer. The aim of this study was to find the stable conformation of the peptide and understanding its key interactions with lipid bilayer. GROMOS force field implemented in GROMACS software, version 4.0 was employed for the molecular dynamics simulations. The results showed that this peptide adopted beta helical structure, in agreement with the previous studies. However, GLC was stable in the trans-membrane state during 20 ns molecular dynamics simulation. The findings of this study proved that the elctrostatic interactions between the polar residues of the peptide and the polar head group of the lipid bilayer are the most important interactions in the insertion of peptide into the membrane. Furthermore, simulation revealed that the hydrophobic interactions play a key role in the stability of GLC peptide in hydrophobic environment of lipid bilayer.

1169-Pos Board B79
Quality Assessment of Predicted Protein Structures by Using Molecular Dynamic Simulations
Jiong Zhang, Jingfen Zhang, Qingguo Wang, Yi Shang, Dong Xu, Ioan Kosztin.
In silico protein structure prediction using efficient fully automated servers continues to remain a challenging problem. While many of these servers can generate near-native structures, the lack of reliable structure quality assessment methods makes the identification of these structures problematic. The most common way of discriminating between predicted structures of a given protein is to employ either knowledge or physics based energy functions. Our recently developed MUFOLD-MD server uses an alternative ranking method of the predicted protein structures by testing their relative stability against gradual heating during all atom molecular dynamics (MD) simulations. We refer to this approach as the MD-Ranking (MDR) method. The MUFOLD-MD server consists of three sequential steps involving structure: generation, refinement and selection. First, by using sequence-profile alignment (e.g., PSI-BLAST) and profile-profile alignment (e.g., HHSearch) methods the query sequence is classified as either ''hard'' or ''easy'' target. For hard targets, models are generated using the Rosetta 3.1 software (ab initio method) and then ranked by using their Rosetta energy score. For easy targets, models are generated with the Multi-Dimensional Scaling (MDS) method and then ranked using the OPUS_Ca scoring function. Next, the structures (only hard targets) are refined by employing the ''relax'' mode in Rosetta 3.1. Finally, the MDR method is used to select the top 5 structures as the output of the server. Our MUFOLD-MD server was tested in both CASP8 and CASP9 competitions. Based on the official CAP8 results, MUFOLD-MD was ranked as number one server in the Free Modeling category.
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1170-Pos Board B80
Identification of ERK2-Substrate Protein Inhibitors via Virtual Screening, Biological Assays and X-Ray Crystallography
Taiji Oashi, Jun Zhang, Kimberly Burkhard, U. Deva Priyakumar, Edvin Pozharskiy, Paul Shapiro, Alexander D. MacKerell Jr.
Extracellular signal regulated kinases (ERK1/2) are involved in signaling events that regulate cell division and proliferation. Hyperactivation of ERK has been implicated in the pathogenesis of many human cancers. The F-site recruitment site (FRS) (L198, H230, Y231, L232, L235, and Y261) as well as common docking (CD) (D316 and D319) and ED (T157 and T158) domain in ERK2 is used to facilitate interactions with substrate proteins. Thus, small molecules targeting FRS and/or CD/ED domain have the potential to modulate ERK2 specific functions, potentially leading to the development of novel therapeutic agents. MD simulations of ERK2 from which structurally diverse conformations were selected were used to identify putative binding sites for low molecular weight compounds in the vicinity of the FRS and CD/ED sites. Identified sites were then targeted in individual database screens of over 1.5 million compounds. Following two levels of database screening, fingerprint based similarity clustering and analysis of physicochemical properties that maximize bioavailability, final compounds for biological assay were selected for each site. Inhibition of ERK2-specific phosphorylation was confirmed and dose-dependency was measured in several cancer cell lines using colony survival assays. Direct binding of active compounds to ERK2 was validated by fluorescence quenching experiments. ERK2 was crystallized in complex with several active compounds, showing binding in the critical site in FRS. These identified compounds provide novel tools to study the biological functions of ERK2 as well as act as lead compounds for the development of novel therapeutic candidates for cancer.

1171-Pos Board B81
Sifting a Massive Virtual Library of Peptide Ligands for an Optimal Binder to a Given Receptor
Gungor Ozer, Denise C. Enekwa, Shi Zhong, Stephen Quirk, Rigoberto Hernandez.
The identification of an optimal protein ligand that binds to a target is a difficult problem because the library contains more than a mole of ligands if only 18 residue sites are allowed to vary across all the naturally occurring amino acids. Such a library is far too large to specify explicitly. Instead, the so-called massive virtual library (MVL) is specified indirectly through a set of class rules. An efficient search method through the MVL, which incorporates the principles of sequence design, protein docking and statistical mechanics, has been recently introduced:* (i) a random sub-library is created according to the user defined pruning criteria, (ii) each member in the sub-library is docked to the target using AutoDock and ranked according to the binding free energies that are calculated with both AutoDock's scoring function and CHARMM non-bonded interaction energies, (iii) using the calculated free energies, Boltzmann weighted probabilities are assigned to each sequence, (iv) the weights are then used to select the next-generation MVL, and (v) iterate back to step (i) using the current MVL. The algorithm concludes when convergence occurs between the results from two subsequent rounds. For an 8 residue peptide design that binds to Deoxyribonuclease I, the convergence is achieved in as few as 16 iterations. The generated sequence was found experimentally to have high binding affinity selectively towards the desired target.
*S. Quirk, S. Zhong, and R. Hernandez, Proteins: Struct. Func. Bioinfo. 76, 693-705 (2009).

1172-Pos Board B82
Mechanisms of Interaction Between Lung Collectins and Influenza a Virus Hemagglutinin
Michael J. Rynkiewicz, Dong Luo, Nancy Leymarie, Erika C. Crouch, Kevan L. Hartshorn, James F. Head, Francis X. McCormack, Martin van Eijk, Joseph Zaia, **Barbara A. Seaton**.
The unpredictability and rapid appearance of new influenza A virus (IAV) strains pose major challenges for global health. Highly virulent strains can evolve quickly through genetic reassortment from animal IAV strains and spread rapidly through populations lacking immunity. Some pandemic IAV strains can kill patients rapidly, before the acquired immune system can respond, underscoring the importance of the innate response. The innate activity of pulmonary surfactant proteins A (SP-A) and D (SP-D) forms a front line defense against inhaled pathogens. SP-D targets IAV through lectin-based recognition of high-mannose glycans attached to specific glycosylation sites on viral hemagglutinin (HA) and neuraminidase (NA). This recognition drives HA binding, NA inhibition, and viral aggregation and neutralization. SP-A and glycosylated SP-D variants utilize different mechanisms involving sialic acid recognition by HA. Our studies aim to determine the molecular basis of these

mechanisms. Since growing crystals of large complexes that are heterogeneously glycosylated like the HA/SP-D or HA/SP-A complexes is difficult, we have undertaken computational studies to model the interactions. Crystal structures of HA, SP-A, and SP-D with small saccharide ligands offer starting points for modeling full length complex type or high-mannose glycosylation using low energy sugar chain conformations derived from solution studies. Further computational approaches are used to obtain an ensemble of potential complex structures. Analysis of these structures can give insights into the mechanisms of inhibition of SP-D and SP-A variants, as well as the specificity of SP-D and SP-A for certain glycoforms of HA. Measurement of the differences in glycosylation site occupation and the glycans residing on HA and SP-A or SP-D is an important adjunct to the molecular modeling. A complete strategy based on liquid chromatography/tandem mass spectrometry analysis is underway to investigate the different glycosite and glycoforms present.

1173-Pos Board B83

Small Molecule HIV-1 Capsid Inhibitor Design using Hybrid Structure Based Methods

Sandhya Kortagere, Navid Madani, Marie K. Mankowski, Amy Princiotto, Kevin Anthony, Luz-Jeannette Sierra, Xiaozhao Wang, David M. Jones, Joel R. Courter, Eric Stavale, Roger Ptak, Amos B. Smith III,, Julio Martín-García, Joseph Sodroski, Simon Cocklin.

Protein-protein interactions (PPI) are fundamental to almost all biological and pathological processes and therefore represent an important class of therapeutic targets that can be utilized against multi-drug resistant pathogens such as HIV-1. The HIV-1 capsid (CA) protein has recently emerged as an attractive target as it performs essential roles, both regulatory and structural in early and late stages of the viral life cycle. Hence, small-molecule inhibitors of capsid assembly would be attractive and novel antiretroviral medications. With the latest crystal structure of the hexameric arrangement of CA monomers, atomic level details of the CA hexamer interface are now available. The HIV-1 capsid shell is composed of ~250 CA hexamers and 12 CA pentamers which are arranged in the form of a fullerene cone. Crystal structure analysis shows a weak association between the monomers and hence the interfaces formed between the N-terminal (NTD) and C-terminal domains (CTD) seem attractive as drug targets. In this study, we targeted the NTD-NTD interface region as a novel PPI to design inhibitors using the hybrid structure based method. Our preliminary results show that we have identified two compounds (CK026 and CK422) that display significant antiretroviral activity against HIV-1. Importantly, these compounds belong to two distinct inhibitory classes: early-stage inhibitors and late-stage inhibitors. Compound CK026 represents the first CA-targeted small molecule that works by disruption of pre-integration events in HIV-1 replication. Further chemical modification has led to identification of other analogs that retain the antiviral activity with improved drug like properties.

1174-Pos Board B84

Protein Loop Modeling using Distance-Guided Sequential Monte Carlo Method

Ke Tang, Jinfeng Zhang, Jie Liang.

Modeling loop regions is an important task for protein structure prediction. We have developed a new loop construction method through efficient sampling with a loop-specific energy function. Based on a new sequential Monte Carlo sampling strategy called Distance-guided Sequential Monte Carlo (dSMC), our method efficiently generate loop conformations with lower energy. To derive the loop-specific energy function, a decoy-based reference state is used with a large set of loop conformations. Our approach is works well in modeling long loops. The average smallest global RMSD for 11 residue loops generated is about 1.5 A. Our method also addresses the challenging problem of multi-loop modeling. As loops often are in spatial proximity and interact with each other, our approach treats these loops simultaneously and sample multiple loop efficiently. As an example, for the protein ribonuclease A (PDB id: 7RSA) which has 3 loops (7+4+5 residues), the global RMSD for the loops with the lowest energy compared to the known structure is only 0.52 A.

1175-Pos Board B85

Structure Based Inhibition of Mitochondrial Aldehyde Dehydrogenase (ALDH2) Activity

Ann C. Kimble-Hill, Hina Younus, Samy Meroueh, Thomas D. Hurley.

Mitochondrial aldehyde dehydrogenase (ALDH2) is an enzyme that participates in multiple metabolic pathways, including the oxidation of toxic biogenic and environmental aldehydes. Our laboratory is interested in developing novel and selective ALDH2 inhibitors. We recently identified and studied three distinct classes of small molecules based on their ability to inhibit ALDH2 activity (esterase, dehydrogenase, and both). These inhibitors were identified by virtual screen which consisted of three steps: 1) docking 800,000 ChemBridge molecules to the substrate cavity in ALDH2; 2) scoring the receptor-ligand complexes while computing the binding affinity; and 3) ranking the top 1,000 compounds using GlideScore to determine the top 250 compounds for study. From this list, 112 compounds were selected for purchase and screened for inhibitory activity at 50 uM. 19 compounds were selected for further evaluation based on their ability to inhibit hALDH2 propionaldehyde oxidation by more than 60%. IC50 values for these compounds were determined using both the dehydrogenase and esterase assays.

We selected 4 compounds with IC50 values less than 20uM for further kinetic and structural studies: I32, I72, I76, and I78. These inhibitors show a competitive inhibition pattern toward varied NAD+ concentrations and either uncompetitive or noncompetitive inhibition towards varied priopionaldehyde concentrations. Based on their structural similarity and x-ray crystallography structures obtained with these compounds bound to the enzyme, fragments were then chosen for studying the mechanism by which they inhibit ALDH2. The next step is to solve the structures of these fragments bound to ALDH2 and develop SAR data for fragment based compound development that will enhance their specificity for and inhibition of ALDH2. This work was supported by NIH R01-AA18123 and NIH R01-AA18123S1.

1176-Pos Board B86

Role of water and G Protein in modulating agonist affinity in GPCRs

Supriyo Bhattacharya, Michiel Niesen, Alfonso R. Lam, Nagarajan Vaidehi.

Predicting accurate ligand poses and ligand selective receptor conformations are imperative in designing efficacious, functionally specific drugs for G-protein coupled receptors. Comparison of the crystal structures of carazolol (inverse agonist), formoterol(agonist), and agonist with G-protein mimic bound β2 Adrenergic Receptor (β2AR) shows that the agonist stabilizes a slightly different conformation when there is no G-protein bound. The G-protein bound receptor state exhibits a high affinity conformation for the agonist. Using the computational method LITiCon, we have calculated the activation pathways for full, partial and inverse agonists of β2AR, which are in agreement with fluorescence intensity lifetime measurements. MD simulations starting from various conformations along the activation pathway (total ~1.5 μs) show that in the absence of G protein, norepinephrine (agonist) stabilizes an intermediate receptor state that is similar to the formoterol bound intermediate state without the G-protein, in agreement with the recent crystal structure of formoterol bound β2AR. Thus coupling to G protein may be needed for stabilizing the fully active state.

Using Liticon method we have calculated the activation pathway of agonist bound adenosine receptor A2A starting from its inactive state. We found that water plays a important role in the docking of the antagonist as well as the agonist.

We recently predicted the structures of D3 dopamine receptor and CXCR4 chemokine receptor as part of the competition for the assessment of computational methods in GPCR modeling (GPCR Dock 2010). The predicted docked poses of both the ligands are in close agreement with the crystal structures. The details of the methods and comparison with the crystal structures will be presented.

1177-Pos Board B87

Biophysical Approaches to Discovery of Novel Antibacterial Leads

Gautam Sanyal.

This presentation will outline biophysical approaches to discovery of antibacterial lead compounds that are designed to address bacterial resistance to many current antibiotics. There are a number of essential enzymes, in key pathways of pathogenic bacteria, which have not been targeted by existing drugs. The potential bacterial selectivity and spectrum of these single gene targets can be validated using structural tools. In addition, inhibiting clinically exploited and validated bacterial enzymes with a different mechanism (e.g., at a different binding site) than those offered by current drugs can be a powerful approach. Target enzymes that have remained unexploited or those that offer alternative binding sites include cell wall biosynthesis and DNA replication enzymes. In our discovery efforts, biophysical studies including X-ray crystallography and NMR spectroscopy are utilized at all stages of lead generation beginning with target selection, validation, selectivity analysis and probing of target binding sites for druggability. This will be exemplified with comparative structures of target enzymes from different pathways. Hits identified through screening of compound and fragment libraries against these targets are progressed towards leads with the help of biophysical and structural analysis of protein-ligand complexes. Development of effective screening assays for enzyme targets requires a thorough understanding of enzyme mechanism. An example will be given of how water LOGSY measurements by NMR explained the mechanism of a multisubstrate cell wall enzyme. Finally, development of a lead series against